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POLLEN DEGENERATION IN MALE-STERILE SUGAR BEETS, WITH SPECIAL REFERENCE TO THE TAPETAL PLASMODIUM[1]

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INTRODUCTION

When male-sterility in sugar beets (*Beta vulgaris* L.) (*4*)[2] is cytoplasmically inherited, completely male-sterile plants bear white, empty anthers. Normal pollen mother cells and normal microspores are produced, but the microspores fail to develop fully and disintegrate by the time the flowers open.

In the semi-male-sterile types, small, nonviable pollen grains are formed but the anthers usually do not dehisce. Sometimes viable pollen is produced by some branches of the inflorescence, and occasionally white anthers and yellow ones are borne within the same flower (*4*).

Pollen abortion, especially in hybrids of plants with different chromosome numbers, usually is the result of abnormal meiotic divisions; in sugar beets, however, the writer observed a different type of pollen degeneration in which the anther tapetum, through the development of a plasmodium, plays an important role.

Tapetal plasmodia and less prominent forms of the same phenomenon are not uncommon and may be regarded as normal in those plants in which they have been described (*6*), but as a pathological condition they seem to have been mentioned but once in literature (*7*). In sugar beets the tapetal plasmodium dominates the pathological picture from its inception to the destruction of the microspores.

MATERIALS AND METHODS

Terminal branches of sugar-beet inflorescences, fixed in Carnoy's fluid, were sent to the writer by F. V. Owen, of the United States Department of Agriculture Sugar Plant Laboratory, Salt Lake City, Utah. The material was taken from beets exhibiting cytoplasmically inherited male-sterility. For comparison, male-sterility not cytoplasmically inherited was also studied. The completely male-sterile and semi-male-sterile forms were derived by Owen "directly or indirectly from the sugar-beet variety U. S. 1 . . . , which was the first of the curly-top-resistant sugar-beet varieties released by the United States Department of Agriculture" (*4*, *p. 423*).

[1] Received for publication February 13, 1947.

[2] Italic numbers in parentheses refer to Literature Cited, p. 197.

The usual methods of dehydrating and embedding in paraffin were employed. The sections were cut 5μ to 10μ thick and stained with Heidenhain's iron-alum haematoxylin and chromotrope.

All photomicrographs were taken on Wratten M plates with B 58 and E 22 filters used singly and in combination.

THE ANTHER TAPETUM DURING NORMAL MICROSPOROGENESIS

Since microsporogenesis in normal sugar-beet flowers has been described in detail elsewhere (*1*), the discussion here will be limited to the behavior of the tapetum, which plays a major role in pollen abortion of male-sterile sugar beets.

A cross section through a young anther lobe (pl. 1, *A*) shows a central strand of dark-staining primary sporogenous tissue surrounded by a series of parietal wall layers. The first periclinal division in the primary parietal cells gives rise to two layers (pl. 1, *A*), which divide once more in a periclinal fashion. The parietal tissue is composed of a tapetal layer, two or more middle layers, and the endothecium, lying just beneath the epidermis. The cells of the endothecium enlarge late in ontogeny and develop the characteristic fibrous thickenings (pl. 1, *F*), which are related to the dehiscence of the anther.

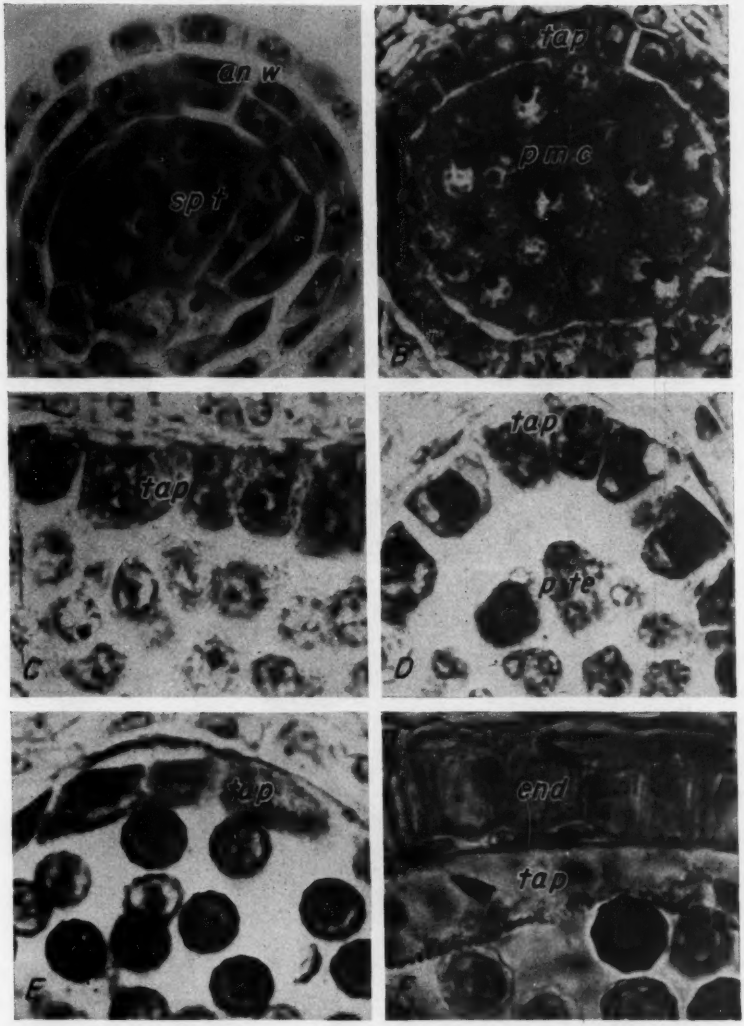
Although there is much controversy in literature concerning the origin of the tapetum, in sugar beets it appears to be derived from the inner parietal wall layer since the primary sporogenous tissue (pl. 1, *A*) is already well differentiated before the second periclinal division in the cells of the parietal tissue takes place. The cells of the young tapetum are nearly isodiametric (pl. 1, *B*). The single nucleus is large and almost fills the cell lumen. Beginning with synizesis, the tapetal cells enlarge radially and become binucleate (pl. 1, *C*). Each nucleus contains one large nucleolus and parietal or diffuse chromatin granules. Some of the tapetal cells may contain compound nuclei (pl. 1, *C*, *D*). The cytoplasm is very dense and stains more intensely than that of the pollen mother cells.

The nuclei and cytoplasm of the tapetum indicate strongly a metabolic relation between tapetum and developing microspores. This active phase is maintained throughout the two meiotic divisions and the liberation of the microspores from the tetrads. With the progressive enlargement of the anthers and the growth of the pollen grains, the tapetal cells become tangentially elongated, the nuclei undergo chromatolysis, and the nuclear substance mingles with the degenerating cytoplasm (pl. 1, *E*). In anthers ready to dehisce the tapetum has completely disappeared or may be seen as a pale narrow band lining the anther cavity (pl. 1, *F*). Remains of small, shrunken nuclei may also be discerned.

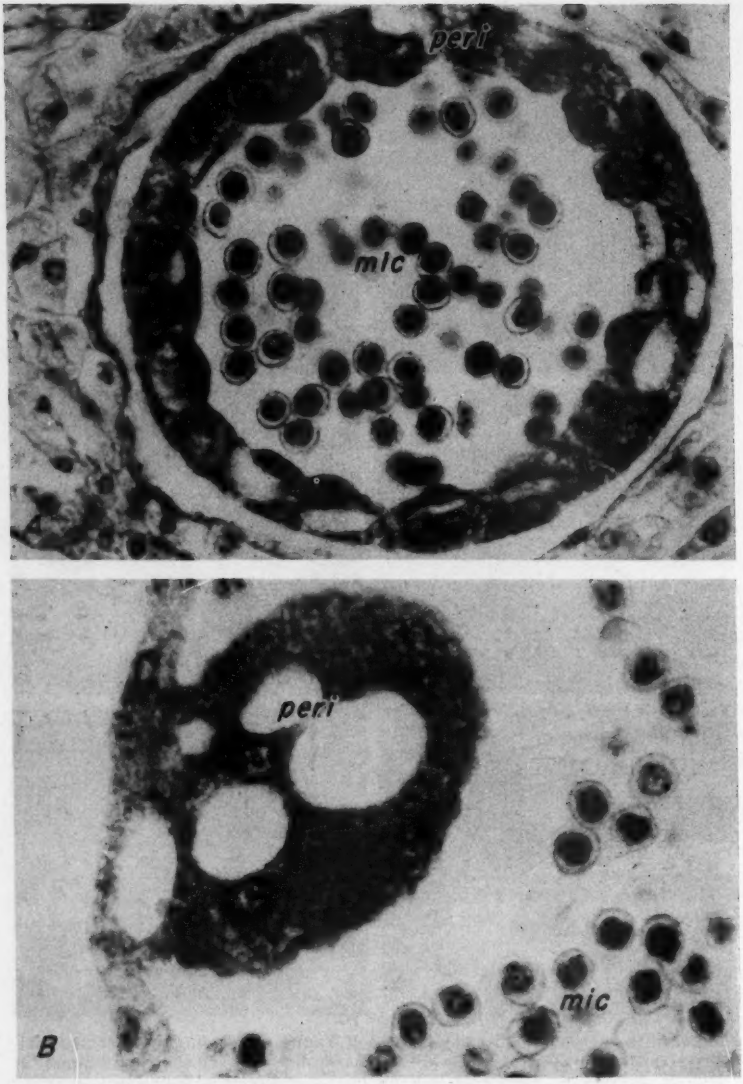
POLLEN ABORTION IN COMPLETELY MALE-STERILE SUGAR BEETS

CYTOPLASMICALLY INHERITED TYPE ASSOCIATED WITH A TAPETAL PLASMODIUM

In many of the lower plants and a number of angiosperms the walls of the tapetal cells break down and allow the protoplasts to coalesce and form a tapetal plasmodium (*5*). This flows in among the immature spores and contributes to the development of their coats.



Normal microsporogenesis in sugar beet: *A*, Young anther lobe with central primary sporogenous tissue and wall layers; *B*, older anther showing differentiation of tapetum; *C*, binucleate tapetum during synizesis of pollen mother cells; *D*, binucleate tapetum during formation of tetrads; *E*, degenerating tapetum and pollen grains with thickened exine; *F*, completely degenerated tapetum, endothecium with wall thickenings, and mature pollen. × 850. *an w*, Anther wall; *end* endothecium; *p m c*, pollen mother cell; *p te*, pollen tetrads; *sp t*, sporogenous tissue; *tap*, tapetum.



Early stages of plasmodial development in anthers of cytoplasmically inherited male-sterile sugar beets: *A*, Early stage in the development of periplasmodium; *B*, somewhat later stage with periplasmodium making pseudopodiumlike incursions into anther cavity; note large nuclei and vacuoles. × 850. *mic*, Microspores; *peri*, periplasmodium.

In the monocots investigated by Clausen (*2*) there are distinct types of plasmodial formations sufficiently different to have taxonomic importance. In many forms the tapetal cells, after the walls separating them have been dissolved, make pseudopodiumlike incursions into the anther cavity; a common plasmodium that surrounds the pollen grains is finally formed. Embedded in the plasmodium are the nuclei of the tapetum. When the pollen grains have developed the exine, the plasma of the plasmodium diminishes and the nuclei begin to degenerate; the chromatin threads break up and become pale; and finally only pollen grains remain.

A case of plasmodium formation in connection with pollen degeneration in *Ranunculus acris* L. has been described by Whyte (*7*, *p. 187*). In the hermaphrodite normal plant there is a considerable interval in the reduction processes in anthers and ovules in a given flower. Where the reduction divisions occur almost simultaneously, the tapetum in the anthers fails to function and cessation of pollen formation results. As a direct consequence, the cells of the tapetum " . . . hitherto normal . . . break apart leaving their usual situation along the wall of the pollen sac; coalescence of several cells may then take place forming large plasmodial masses . . . the whole occupying in many instances the greater part of the pollen sac." Here then is an instance where a normally useful plasmodium causes irreparable injury to the pollen.

The cytology of meiosis in male-sterile sugar beets agrees in every detail with that in normal sugar beets. The young microspores may still be associated in the tetrads when the cells of the tapetum begin to behave in an abnormal manner. The boundaries of the tapetal cells break down, and the contents flow together to form a periplasmodium (pl. 2, *A*).

From its early development to its degeneration the cytoplasm of the plasmodium is very dense and contains conspicuous vacuoles. The nuclei are large (pl. 2, *B*) and often are bunched in great numbers (pl. 3, *A*), suggesting an increase over the number present in the original cellular tapetum. The nuclei are round, narrow oval, or sometimes lobed. Most of them contain one large nucleolus and peripheral chromatin granules of various sizes. Some nuclei appear definitely compound, containing two or three large nucleoli.

Most of the young microspores appear normal. Each microspore has a large nucleus embedded in dense cytoplasm which, perhaps as a result of fixation, is slightly shrunken away from the spore wall (pl. 2, *B*). There is a single large nucleolus. In some of the dark-staining microspores there may also be seen large chromatin granules resembling typical chromocenters. Some of the microspores have become pycnotic and stain black. In a few of these a dark-staining nucleus containing large chromatin granules is discernible under intense illumination. The spore wall is very thin, and it fails to thicken even late in ontogeny. Some microspores are represented by spore membranes only, all cell contents having disappeared.

The plasmodial jacket increases in width unequally. Here and there it bulges prominently into the anther cavity (pl. 2, *B*). These broad pseudopodiumlike incursions never become detached from the main jacket and never surround the microspores as they do in plants in which the appearance of a plasmodium is considered a part of normal microgenesis.

With the growth of the plasmodium the microspores are crowded into the narrowing lumen of the anther (pl. 3, *A*). So far there has been no noticeable structural change within the microspores except that additional ones have become pycnotic. There is no increase in thickness of the spore membrane.

After the plasmodium has attained maximum development, it begins to degenerate. The cytoplasm is first affected. It becomes coarsely alveolar (pl. 3, *B*) and then stringy (pl. 4, *B*). In its alveolar state it stains rather light; but later it retains the stain more tenaciously (pl. 4, *A*), and the cytoplasmic strands finally stain black (pl. 4, *B*). The large nuclei of the plasmodium are at first little affected (pl. 3, *B*), but with increased destruction of the cytoplasm they also exhibit signs of degeneration. At the beginning there is an increase in the number of peripheral chromatin granules in the nucleus and a deeper staining reaction (pl. 4, *A*), but they retain a certain degree of organization even after the complete disorganization of the cytoplasm.

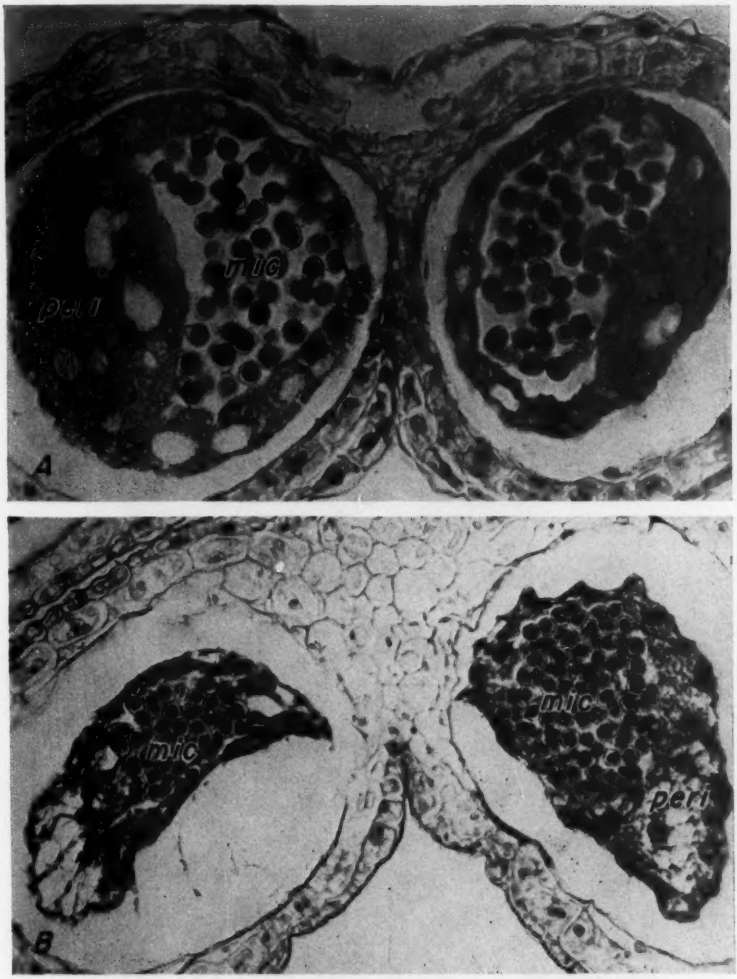
According to Kostoff (*3*), evidently only the cytoplasm possesses the ability of self-regulation, being able to extract foreign substances and those not of immediate use and to deposit them in vacuoles. The nucleus is always more susceptible to foreign agents, and it is killed by poison before the cytoplasm is irreparably injured. When the plasmodium in male-sterile sugar beets is in its ascendancy, the large vacuoles, which are a notable feature of the organization of the plasmodium (pl. 2, *B*), may be places of storage for excess waste products resulting from unusual metabolic activities. These waste products may be released with the advent of plasmodial degeneration and may hasten the destructive processes which begin after the peak of plasmodial development has been reached. According to Kostoff, one would expect an early destruction of the nuclei, but the cytoplasm deteriorates before there are recognizable pathological changes in most of the nuclei. Nevertheless it seems that with the disappearance of the protecting vacuoles the cytoplasm would become more susceptible than the nuclei.

The microspores continue to show little damage even though they become increasingly compressed and in their entirety take on a honeycomb structure (pl. 5). With the complete disintegration of the plasmodium the microspores are finally destroyed. The anther cavity becomes empty except for a strand of blackened matter, which occasionally still shows evidence of cellular structure.

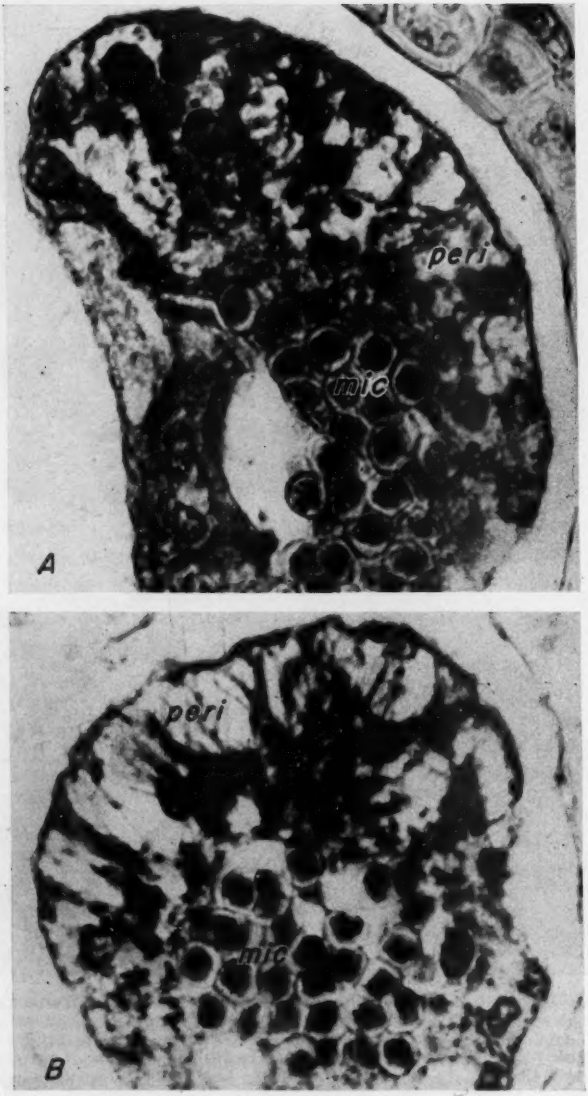
CYTOPLASMICALLY INHERITED TYPE ASSOCIATED WITH A CELLULAR TAPETUM

Pollen abortion not associated with a tapetal plasmodium frequently occurs; but while the flowers of a cluster may exhibit both types of degeneration, within a given flower only one type prevails.

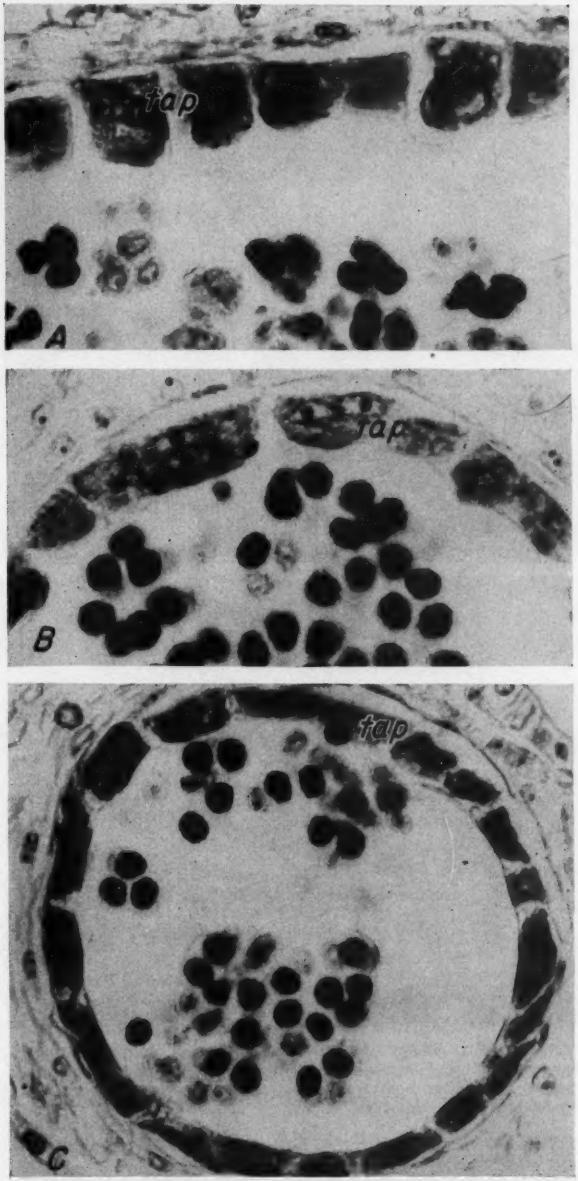
In cases not involving a plasmodium, the tapetum remains cellular. It forms either a narrow lining (pl. 5, *A*, *B*) in which the individual cells may be stretched tangentially (pl. 5, *B*) or a very broad jacket with the cells meeting almost in the center. The former type is more common. Under low magnification the anther cavity appears large, round, and partly filled with microspores in various stages of degeneration (pl. 5, *C*). The tapetal cells are completely filled with fine gran-



Comparative stages of plasmodial development in anthers of cytoplasmically inherited male-sterile sugar beets. *A*, Periplasmodium at peak of development; note that some of the microspores are normal, while others are degenerate. × 450. *B*, Degeneration of plasmodium more advanced in the left anther lobe than in the right one. × 385. *mic*, Microspores; *peri*, periplasmodium.



Advanced stages in degeneration of plasmodium in cytoplasmically inherited male-sterile sugar beets. *A*, Degeneration in anther plasmodium; note that some of the plasmodial nuclei stain black and that the microspores in the center are compressed in such a way as to resemble a honeycomb cellular structure. *B*, Final stage in degeneration of plasmodium; the cytoplasm has become stringy, the nuclei are disorganized and black, and the microspores in the center are compressed. × 850. *mic*, Microspores; *peri*, periplasmodium.



Types of cellular tapetum and pollen degeneration in anthers of cytoplasmically inherited male-sterile sugar beets. *A*, Microspores already degenerated in tetrads. *B*, Most of the microspores degenerated and the tapetal nuclei flattened tangentially. × 850. *C*, Only relatively few microspores present, more than half having degenerated while still in tetrads. × 700. *tap*, Tapetum.

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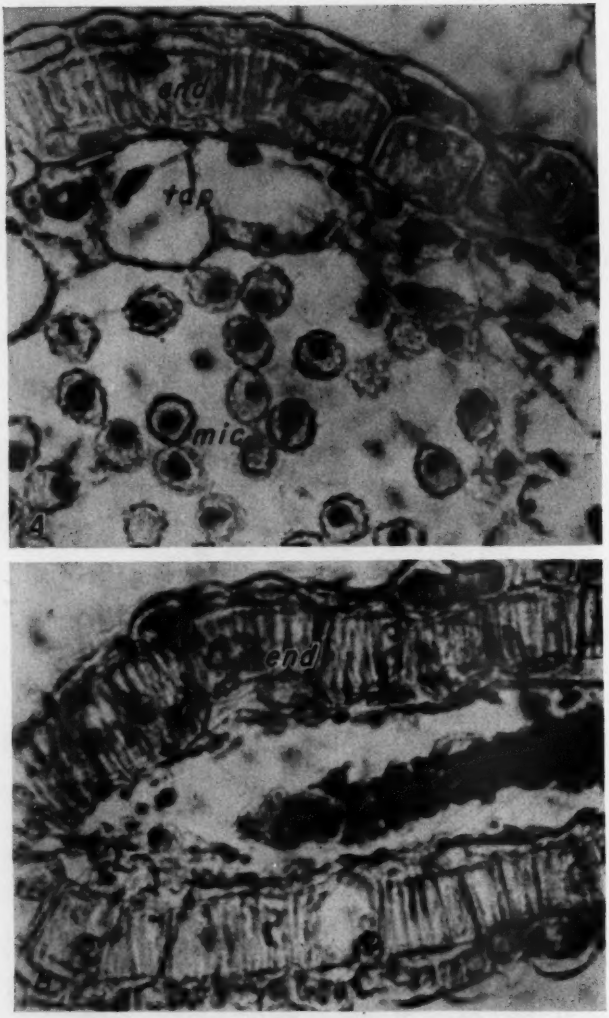


Pollen degeneration in noncytoplasmically inherited male-sterile sugar beets: *A*, Anther with cellular tapetum and fully developed endothecium; *B*, Anther with tapetum and pollen completely degenerated. × 850. *end*, Endothecium; *mic*, microspores; *tap*, tapetum.

ular cytoplasm, in which the nuclei are barely discernible. Vacuoles, which are a prominent feature of the plasmodium, are wanting. The nuclei are small or medium large and usually compressed tangentially (pl. 5, *B*). The tapetal cells at the ends of the anther are shorter, and the nuclei are larger and almost spherical. Most nuclei have a very large nucleolus and a varying number of chromatin granules; some have several nucleoli and are definitely compound (pl. 5, *A*).

Some of the young microspores appear normal and look like those developing in association with the periplasmodium, but the majority have become pycnotic and stain black (pl. 5, *B*, *C*).

Degeneration of the microspores begins rather early, sometimes before they are liberated from the tetrads (pl. 5, *A*). The affected microspores, including those still in tetrads, are completely black, but under intense illumination a nucleus is still discernible in some of them. The outer margin of the blackened microspore is finely serrate or prickly and slightly retracted from the thin, hyaline spore wall.

Progressive degeneration of nuclei and cytoplasm in the tapetal cells is like that observed in normal microgenesis, so that it is difficult to ascribe to the tapetum any harmful influence on the developing microspores. But whatever the cause is, the effect is swift and lethal and there are seldom intermediate stages. Death often overtakes the spores while they are still in the tetrads, and those that are affected later show little or no growth. The cells of the endothecium remain small and fail to develop the characteristic wall thickenings.

NONCYTOPLASMICALLY INHERITED TYPE OF MALE-STERILITY

Anthers from male-sterile plants of the noncytoplasmically inherited type develop the endothecium normally (pl. 6), probably because degeneration of the microspores is initiated later in ontogeny and proceeds more slowly so that the anther wall has had no time to mature. The tapetum of such anthers always remains cellular, but the cells composing it enlarge greatly (pl. 6, *A*). The microspores enlarge slightly and even develop a thin exine. Cytoplasm and nuclei stain weakly and show signs of degeneration. In some microspores the contents are blackened and contracted into a spherical mass. At a later stage the anthers collapse; the anther cavity appears empty except for a dark strand of disintegrating microspores (pl. 6, *B*).

POLLEN DEGENERATION IN SEMI-MALE-STERILE SUGAR BEETS

According to Owen (*4*), the semi-male-sterile types apparently carry the same type of cytoplasm as that carried by completely male-sterile sugar beets, but their appearance is modified by one or more Mendelian factors. Environmental conditions have a marked influence on the expression of the semi-male-sterile condition, and classification for degree of semi-male-sterility may be subject to considerable variation.

A characteristic feature of pollen sterility in semi-male-sterile sugar beets is the lack of uniformity in the pathological picture even within a single flower (pl. 7, *A*). Some anthers are affected so completely that even the anther wall is destroyed, whereas others of the same flower are almost normal. In some anthers the tapetal cells have enlarged radially so greatly as to occlude the anther cavity. These cells

(pl. 7, *B*) have in the cytoplasm large, round nuclei and vacuoles like those of the periplasmodium. This type of tapetum is sometimes unilateral, prominent on one side and completely lacking or underdeveloped on the other. The microspores within such anthers show various stages of degeneration from a nearly normal condition to advanced pycnosis.

The anthers of many flowers appear normal except that the pollen grains, though uniform, are relatively small. Pollen grains in others approach normal size, but many of them are diseased (pl. 7, *C*).

Plasmodia apparently are not formed in anthers of semi-male-sterile sugar beets, but the large nuclei and cytoplasmic vacuoles in the radially elongated tapetal cells closely resemble those of the plasmodium.

DISCUSSION

What are the causative factors in the development of the periplasmodium and why is it restricted to sugar beets that exhibit cytoplasmically inherited male-sterility? The cytology of the young plasmodium suggests hypermetabolic activity, but not to the benefit of the developing microspores. The plasmodium seems to develop with structural continuity even though it makes pseudopodiumlike incursions into the anther cavity. What causes the precipitous catabolism after a developmental peak is attained is a moot question. It may be exhaustion of food and nutrient reserves or a sudden release of metabolic waste products presumably stored in the large vacuoles. Degeneration processes are more noticeable here than in normal plasmodia that help nourish the microspores and are themselves finally absorbed.

Still more puzzling is the question of selectivity which incites or inhibits the development of the plasmodium in different flowers of the same plant or the same flower cluster. Quite likely small inner environmental influences suffice to establish the choice, and once the impetus is given it carries on under its own momentum.

The anther plasmodium may prove to be a valuable tool in the elucidation of cytoplasmic inheritance in its pure form or in association with Mendelian factors, especially if used on genetic material grown in a controlled environment.

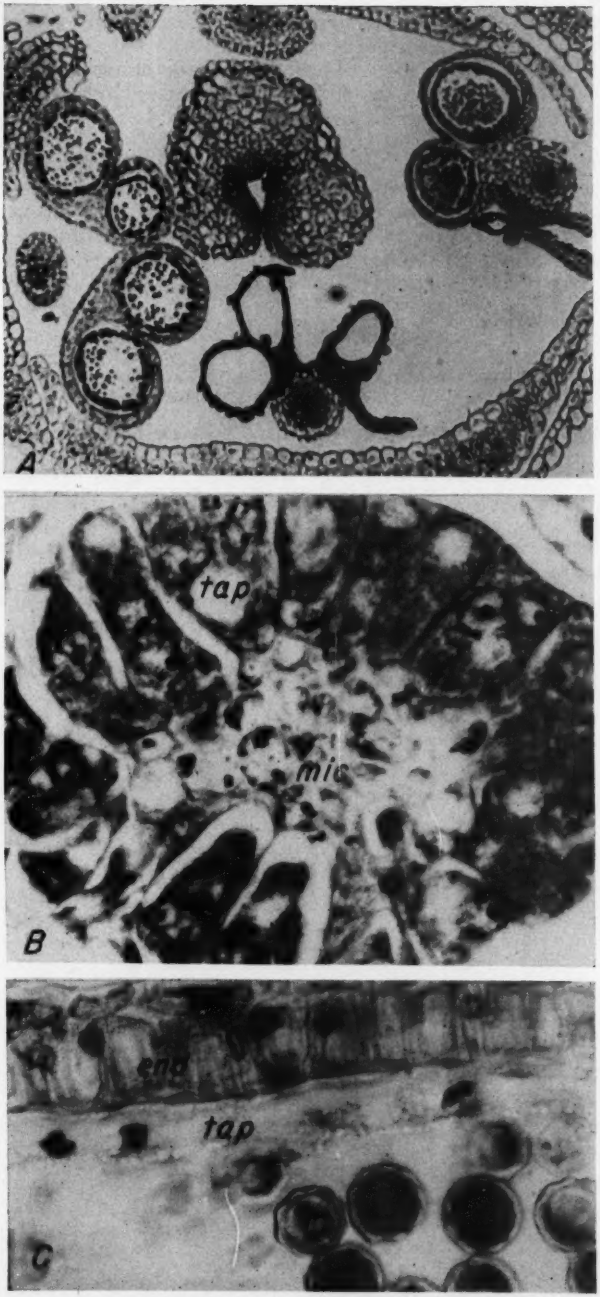
SUMMARY

Pollen abortion in anthers of sugar beets with cytoplasmically inherited male-sterility is associated with either a periplasmodium or a cellular tapetum. Both types may occur within a flower cluster but not within a single flower. The presence of a plasmodium somewhat delays pollen abortion, but where the tapetum remains cellular some microspores are destroyed while still in tetrads and there are seldom intermediate stages of significant duration.

The anatomical picture of pollen degeneration in semi-male-sterile

EXPLANATORY LEGEND FOR PLATE 7

Pollen degeneration in cytoplasmically inherited semi-male-sterile sugar beets. *A*, Cross section of flower; note that both contents and anther wall of one anther are completely destroyed. × 90. *B*, Cross section of an anther showing the circle of hypertrophied tapetal cells enclosing a few degenerated microspores in center. × 850. *C*, Diseased, black-staining pollen grains and normal ones side by side in the same anther. × 850. *end*, Endothecium; *mic*, microspores; *tap*, tapetum.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

sugar beets shows much variation and departs considerably from that exhibited by the completely male-sterile forms. The effect is at once severe and localized, involving at times both microspores and anther wall. A plasmodium never develops, although the cells of the tapetum become hypertrophied and may fill the anther cavity. Such hypertrophied cells have large nuclei and vacuoles like those in the periplasmodium.

In anthers from male-sterile sugar beets of the noncytoplasmically inherited type degeneration of the microspores seems to be delayed. Here the anthers, while showing only blackened contents, have a mature endothecium with fibrous wall thickenings well developed. The tapetum remains cellular. The cells composing it enlarge greatly, but they disintegrate at the time the microspores are destroyed.

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GROWTH RESPONSES OF TOBACCO SEEDLINGS IN ASEPTIC CULTURE TO DIFFUSATES OF SOME COMMON SOIL BACTERIA[1]

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INTRODUCTION

The conditions that cause the development of symptoms of frenching in tobacco (*Nicotiana tabacum* L.) plants are unknown. Low nitrogen,[3] low acidity, and excessive moisture[4] have at times been held to be contributing factors. Nevertheless soil from fields of severely frenched plants was often incapable of producing this morphological abnormality in the greenhouse. Abnormal plants transplanted to fresh soil almost always resume normal growth. The disorder is nontransmissible. Spencer[5] and others have therefore suggested that a labile organic substance (toxin) sometimes present in soils may be the causative agent in this disease.

Bacterial interrelations as revealed through studies with antibiotics seem to indicate the possibility that similar diffusates capable of a direct action on plants may also accumulate in soil under certain conditions. Data published by the writer[6 7] afford direct evidence that low concentrations of an organic substance, the amino acid *dl*-isoleucine, in contact with the roots of tobacco seedlings in aseptic cultures may bring about morphological abnormalities resembling those of frenching.

The present studies were therefore undertaken to determine whether any of the more common species of soil bacteria form diffusates capable of causing abnormal alterations in gross morphology of tobacco plants. The seedlings were aseptically grown in a constant environment. The growth medium selected was a slightly acid mineral agar containing a trace of Bacto-peptone. With or without sucrose

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[2] The writer wishes to express his appreciation for the courtesy shown him by N. R. Smith, Division of Soils, Fertilizers, and Irrigation, Bureau of Plant Industry, Soils, and Agricultural Engineering, who selected and furnished cultures of all the bacteria used.

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this medium was very favorable for growth of tobacco seedlings, but unfavorable for the development of the bacteria studied.

EXPERIMENTAL PROCEDURES

Seedlings of Xanthi Turkish tobacco were grown aseptically on 50 cc. of a mineral agar of pH 5.5 containing 200 p. p. m. of Bactopeptone in 200-cc. Erlenmeyer flasks at 25° C. in continuous light. Illumination of about 500 foot-candles was furnished by 3,500° white fluorescent lamps. Other details of the procedure have been given in a previous publication.[8]

Bacterial inoculation of aseptic cultures was made with a straight, sharpened needle, the stab being located ¾ to 1½ inches from the stem of the seedling. The age of the seedlings at the time of inoculation varied, the range being about 3 to 7 days.

GROWTH RESPONSES TO VARIOUS BACTERIAL SPECIES

The abnormalities in form of Xanthi Turkish tobacco seedlings grown in flasks inoculated with 27 species of bacteria are tabulated in table 1. Inoculations were made 1 inch from stems of seedlings in aseptic culture 1 week after the plant was inserted. Plants were harvested 3 weeks later. Of the 30 bacterial cultures 19 form nitrite from nitrate; 2 of the organisms, *Erwinia carotovora* and *Phytomonas tumefaciens*, are plant pathogens. Two strains each of *Agrobacterium radiobacter*, *Escherichia coli*, and *Pseudomonas fluorescens* are also included.

Abnormalities in growth of tobacco seedlings were caused by 10 of the 25 nonpathogenic species of bacteria in the absence of sucrose. *Erwinia carotovora* also brought about abnormalities in gross morphology. Symptoms with various species differed as a rule, but not always. Those with *Bacillus cereus* and *Erwinia carotovora* appeared identical. On the other hand, one strain of *Agrobacterium radiobacter* produced symptoms of injury in tobacco but the other did not. Symptoms included narrow, strap, cupped, rim-bound, and rim-rolled leaves and chlorosis and epinasty. Cupped and rim-bound leaves might be concave up or down. Chlorosis varied from general to reticular and gave some indication of association with the capacity for nitrite formation from nitrate by bacteria. Chlorosis did not occur as frequently with bacteria incapable of forming nitrite. The addition of 2 percent of sucrose to the medium led to a loss in "hormonoid" [9] action in 7 species of bacteria and a gain in 3 species. In no instance did the seedlings show any indication of root injury, nor were the bacterial colonies in actual contact with the roots.

[8] See footnote 7, p. 199.

[9] A hormone is defined as an organic compound formed by an organism that serves to regulate its cellular interrelations. The chemical compounds in the diffusates from these bacteria may or may not be identical in composition with those having a similar action in the tobacco plant. Pending proof of identity, the use of the term "hormonoid" (hormonelike) is suggested. The synthetic growth-regulating substances would fall into this category.

TABLE 1.—*Symptoms of injury shown by Xanthi Turkish tobacco seedlings grown in aseptic cultures inoculated with 25 nonpathogenic soil bacteria and 2 phytopathogenic bacteria*

[Seedlings grown for 3 to 7 days in aseptic cultures before they were inoculated; discarded when about 4 weeks old]

Culture No.	Organism		Nitrite produced [2]	Symptoms produced when—	
	Name	Strain No. [1]		Sucrose absent	Sucrose present
1	*Aerobacter aerogenes* (Kruse) Beijer.	104–48	+	Reticular chlorosis	Reticular chlorosis.
2	*Aerobacter cloacae* (Jordan) Bergey et al.	108–98	+	White leaves; dwarfed plants.	
3	*Agrobacterium radiobacter* (Beijer. and Van Delden) Bergey et al.	96–433$_1$	0		
4	do	98–189	+	Leaf-tip chlorosis; dwarfed plants.	
5	*Bacillus brevis* Mig.	751	+		
6	*Bacillus cereus* Frankland and Frankland.	342$_1$	+	Reticulated (chlorosis) and scalloped strap leaves.	
7	*Bacillus circulans* Jordan	358	+		
8	*Bacillus megatherium* D By	343$_1$	0		
9	*Bacillus pumilus* Gottheil	272	0	Dark, narrow, down-cupped leaves with uprolled rims.	
10	*Bacillus Sphaericus* Neide	348$_1$	0		
11	*Bacillus subtilis* Cohn emend. Prazmowski.	231	+		Light-green, dwarfed plant.
12	*Bacterium denitrificans* Lehmann and Neumann.	100–12$_1$	+		
13	*Bacterium globiform* Conn	168–110	?		
14	*Chromobacterium violaceum* (Schroet.) Bergonzini.	114–103	+		Very faint mottle.
15	*Corynebacterium simplex* Jens.	140–47	?	Leaf epinasty, rim uproll; terminal bud killed.	Leaf epinasty; down-cupped leaves.
16	*Corynebacterium tumescens* Jens.	141–45	?	Dark, narrow, down-cupped leaves with uprolled rims.	
17	*Erwinia carotovora* (L. R. Jones) Holland.	171–120	+	Reticulated (chlorosis) and scalloped strap leaves.	
18	*Escherichia coli* (Mig.) Castellani and Chalmers.	102–80	+	White leaves and bud; dwarfed plant.	
19	do	107–201	+	Reticular chlorosis	
20	*Micrococcus luteus* (Schroet.) Cohn.	101–244	+		
21	*Phytomonas tumefaciens* (E. F. Sm. and Town.) Bergey et al.	172–102	?		
22	*Proteus vulgaris* Hauser	1354–44	+		
23	*Pseudomonas aeruginosa* (Schroet.) Mig.	110–98	+	White leaves and bud; dwarfed plant.	General chlorosis.
24	*Pseudomonas fluorescens* Mig.	74–210	+		
25	do	112–277	+		White leaves and bud; dwarfed plant.
26	*Pseudomonas ovalis* Chester	77–180	0		
27	*Pseudomonas schuylkilliensis* Chester.	75–180	0		
28	*Sarcina flava* D By	68–178	0		
29	*Serratia lactorubefaciens* (Gruber) Bergey et al.	139–60	+		
30	*Serratia marcescens* Bizio	175–265	+	Reticular chlorosis	Mottled.

[1] Number of N. R. Smith, Division of Soils, Fertilizers, and Irrigations.
[2] Data furnished by N. R. Smith.

The types of morphological abnormalities resulting from diffusates from bacterial colonies are illustrated in figures 1 to 4. A frenching-like reticular chlorosis due to the presence of *Serratia marcescens* is shown in figure 1, *A;* epinasty associated with *Corynebacterium simplex* in figure 1, *B;* chlorosis associated with *Aerobacter aerogenes* and resembling that caused by iron deficiency in figure 1, *C.* The seedlings inoculated with *Aerobacter aerogenes* (fig. 2, *A*), *Corynebacterium simplex* (fig. 2, *B*), and *Pseudomonas aeruginosa* (fig. 2, *C*) illustrate symptoms obtained in the presence of sucrose, namely, yellow bud, epinasty, and a uniform, minus-sulfur type of chlorosis.

Figure 3 shows cultures inoculated with *Corynebacterium tumescens* (*A*), *Erwinia carotovora* (*B*), and *Bacillus cereus* (*C*), respectively. The first (*A*) shows epinasty and long, narrow leaves, whereas the symptoms with *E. carotovora* and *B. cereus* are almost identical in appearance. The last two show a well-defined, reticular chlorosis and narrow strap leaves with lobed or scalloped edges. The appearance of reticular chlorosis preceded that of strap leaves.

Cultures inoculated with *Bacillus pumilus* (No. 272) are shown in figure 4. No sucrose was added to the flask illustrated in *A*, but that in *B* contained 2 percent of sucrose. Inoculation took place after 3 days' growth of the seedlings and at the very margins of the agar. Epinasty, marginal leaf roll, narrow leaf, and cupped leaf are visible.

GROWTH RESPONSES TO VARIOUS STRAINS OF BACILLUS PUMILUS

Further tests were made with 29 strains of *Bacillus pumilus* to determine the range of symptoms to be expected in a single species. The symptoms obtained in these tests, which were made in the absence of sucrose, are listed in table 2. Eighteen of the strains led to no

TABLE 2.—*Variations in symptoms of abnormality in Xanthi Turkish tobacco with different strains of Bacillus pumilus after 28 days' growth* [1]

Culture No.	Strain No.	Symptoms of injury
1	A21	
2	A32	
3	A35	Epinasty; concave-up leaves with marginal uproll; short stem.
4	A170	Epinasty; narrow leaves with marginal uproll; tall stem.
5	A654	Dark, narrow, hooked leaves with marginal uproll and concave-down leaves.
6	A704	
7	236	Narrow, concave-down leaves with marginal uproll.
8	266	Narrow leaves with marginal uproll; tall stem.
9	307	Epinasty; concave-down leaves with marginal uproll.
10	331	Narrow leaves with marginal uproll; tall stems.
11	333	Epinasty; concave down leaves; short stem.
12	334	
13	345	Reticular chlorosis.
14	355	
15	576	
16	577	
17	620	
18	629	
19	630	
20	637	Leaves with marginal uproll.
21	657	
22	706	
23	707	
24	724	
25	725	
26	734	Retarded growth.
27	735	
28	788	
29	1034	

[1] Flasks inoculated on extreme periphery when seedlings were 3 days old.

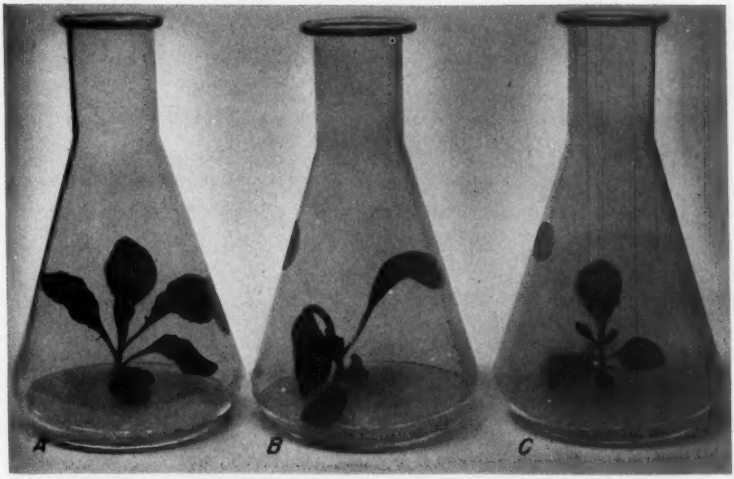

FIGURE 1.—Seedlings of Xanthi Turkish tobacco in aseptic culture after inoculation of agar 1 inch from stems with nonpathogenic soil bacteria: *A, Serratia marcescens; B, Corynebacterium simplex; C, Aerobacter aerogenes.* Note the reticular chlorosis in *A*, the epinasty of leaves and death of terminal bud in *B*, and the chlorosis resembling that caused by iron deficiency in *C*.

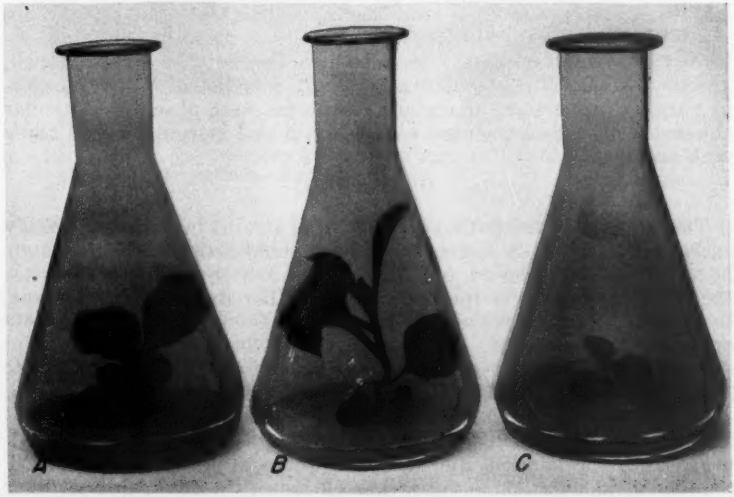

FIGURE 2.—Seedlings of Xanthi Turkish tobacco in aseptic culture with 2 percent of sucrose after inoculation of agar 1 inch from stems with nonpathogenic soil bacteria: *A, Aerobacter aerogenes; B, Corynebacterium simplex; C, Pseudomonas aeruginosa.* Note yellow bud in *A*, cupped leaves with uprolled rims in *B*, and whiteness of plant in *C*.

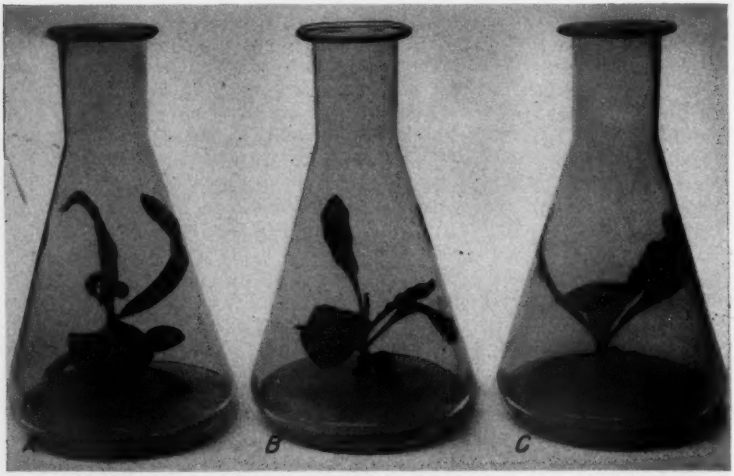

FIGURE 3.—Seedlings of Xanthi Turkish tobacco in aseptic culture after inoculation of agar at margin with various bacteria: *A, Corynebacterium tumescens; B, Erwinia carotovora; C, Bacillus cereus.* Note narrow leaves and epinasty in *A* and reticular chlorosis and scalloped strap leaves in *B* and *C.*

symptoms of abnormality in the seedlings. Some seedlings in inoculated flasks appeared even more vigorous than the controls. Symptoms associated with the other 11 strains ranged from simple retarded growth to epinasty, concave-up leaves with marginal uproll, and short stem. Intermediate abnormalities included these symptoms in varying proportion, or certain symptoms were absent. Reticular chlorosis only was associated with 1 strain and marginal uproll alone with another.

DISCUSSION

The number of nonpathogenic bacterial strains in soils is probably exceedingly great. A discussion of hormonoid actions brought about by their diffusates based on studies with less than 60 strains must therefore necessarily be limited in scope. The data suffice, however, to demonstrate in a conclusive manner that such hormonoid effects on tobacco can take place under appropriate conditions with bacterial species of general occurrence. Isolation and study of these compounds of bacterial activities may well prove of importance in many respects.

It was found in miscellaneous unreported experiments that the extent of the alterations in form of seedlings seemed to increase with nearness of the point of inoculation of the agar to the stem and the number of inoculations. Other known factors were size of seedling and the presence or absence of sucrose. Sometimes, however, severity of symptoms varied for no known reason, as in flasks inoculated with *Serratia marcescens.* Reproduction of symptoms occurred regularly with *Corynebacterium simplex* and *Bacillus pumilus.* The type of hormonoid action was not entirely specific for any one species, but

FIGURE 4.—Seedlings of Xanthi Turkish tobacco in aseptic culture after inoculation of agar at margin with *Bacillus pumilus* (No. 272): *A*, Without sucrose; *B*, with sucrose. Note epinasty and death of terminal bud in *A* and epinasty and narrow, hooked, rim-rolled leaves in *B*.

appeared to vary with the strain. In one instance *Erwinia carotovora* and *Bacillus cereus* produced identical symptoms.

Growth of the bacteria used was usually limited to the inoculation stab and did not appear to interfere with root growth. As a rule, bacterial growth was very scant and the colony was difficult to locate on the unfavorable medium employed. The roots in all cases appeared normal. In many instances morphological abnormalities appeared in the seedlings when no roots were in the immediate vicinity of the bacterial colony.

Proof of the existence of hormonoid responses to bacterial activity in the soil is regarded as an important consideration in elucidation of the cause of frenching in tobacco plants. The basic cause of this profound change in morphology in field plants is unknown, although the alteration is no greater than that effected by soluble metabolic products of some of the bacteria observed in aseptic culture. It might therefore be surmised that frenching is due to a similar action of metabolites from one or more bacterial forms. Hormonoid accumula-

tion in soil probably requires specific environmental conditions. As already mentioned [10] a close approximation of the symptoms of frenching can be obtained with *dl*-isoleucine.

SUMMARY

Xanthi Turkish tobacco seedlings were grown aseptically on 50 cc. of a mineral-agar solution containing 200 p. p. m. of Bacto-peptone in 200-cc. Erlenmeyer flasks at 25° C. with 500 foot-candles of continuous light. Inoculations (stab) of the agar at a distance from the stems of the seedlings with about 60 species and strains of presumably nonpathogenic soil bacteria led in several cases to alterations in gross morphology of the seedlings. These hormonoid effects included various types of chlorosis that simulated various mineral deficiencies, epinasty, cupped, narrow, and strap leaves, and leaves with lobes, hooked tips, and rim roll. Symptoms of abnormality were correlated with distance and number of bacterial colonies, age of seedlings, and bacterial strain. An analogy is drawn between the hormonoid effects of the diffusates from certain common soil bacteria and those obtained in frenching of tobacco.

[10] See footnote 7, p. 199.

USE OF LANOLIN AND OTHER UNGUENTS FOR IMPROVING BUDDING IN HEVEA RUBBERTREES[1]

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INTRODUCTION

In a program of top working mature trees of Para rubber (*Hevea brasiliensis* (H. B. K.) Muell. Arg.) at the United States Plant Introduction Garden, Coconut Grove, Fla., considerable difficulty was encountered in finding a satisfactory dressing for treating wounds made by the removal of large branches or of the entire heads of trees. Success reported for the use of lanolin [2] for the treatment of wounds of oaks led in 1945 to trials of the material on *Hevea* wounds. At this Garden *Hevea* is grown on the deeper areas of soil tentatively referred to as part of the Rockdale series.[3]

USE OF LANOLIN MIXTURE ON TREE WOUNDS

Direct application of lanolin to wounds on *Hevea* rubbertrees in full sun proved unsatisfactory as the lanolin quickly melted and ran down the treated branches or was greatly absorbed by the wood or the adjacent bark. To overcome this difficulty beeswax was added to the lanolin in equal parts. This combination was stiffer than lanolin and had to be applied with a brush after being melted in a grafting-wax pot. Even this mixture had a tendency to run on very hot, sunny days and to crack somewhat on cool days. An improvement was made by coating the wound with the lanolin-beeswax mixture and over this applying one or two thicknesses of cheesecloth, which were then covered with the mixture. The cheesecloth held the mixture in place, prevented cracking, and prolonged the sealing effect. The exposed wood of wounds treated in this manner did not become discolored, as the mixture apparently excluded attacking fungi, and cambial growth was much more rapid than on untreated wounds or on those treated with ordinary paint or several of the asphalt-base wound paints.

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[1] Received for publication November 22, 1946.

[2] UNITED STATES FOREST SERVICE ALLEGHENY FOREST EXPERIMENT STATION. QUICK HEALING OF TREE WOUNDS. Allegheny Forest Expt. Sta. Ann. Rpt. 1943, pp. [24]–[25], illus. 1943. [Processed.] [Review in Horticulture 22: 293. 1944.]

[3] HENDERSON, J. R. THE SOILS OF FLORIDA. Fla. Agr. Expt. Sta. Bul. 334, 67 pp., illus. 1939. On p. 54 of this bulletin the Rockdale series is described as "oolitic limestone with numerous small surface cavities filled with red or reddish-brown sandy loams and silt loams or gray to grayish-brown sands to loamy sands."

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USE OF LANOLIN MIXTURE ON TOP-WORK BUDDINGS

The fact that lanolin protected or stimulated the cambium of wounds on rubbertrees and preserved the wood suggested the possibility that it might also be of benefit in budding operations in *Hevea* nurseries or in top working mature trees. In *Hevea* nurseries many budders have observed that high percentages of buds that seemingly were in excellent condition when unwrapped died during the succeeding 1- or 2-week interval before the tops of the stock plants above them were cut off to force the dormant buds into growth. Experience in

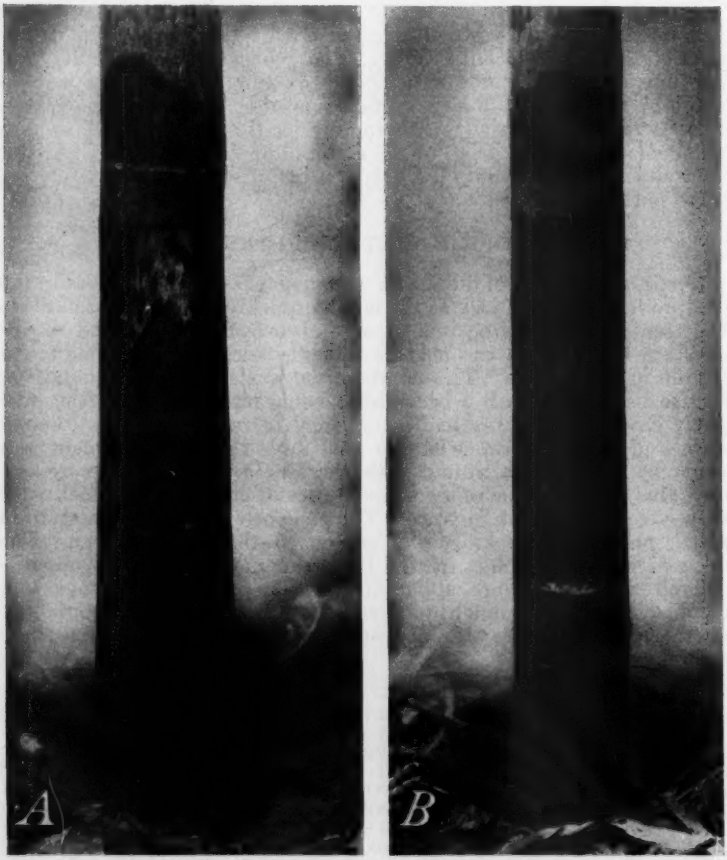

FIGURE 1.—*A*, Untreated budding of *Hevea* rubbertree, 8 days after being unwrapped. Note that the exposed cambium surrounding the bud patch is dead and the wood weathered and discolored; although the bud patch is in good condition, it was dead when examined 12 days later. *B*, Bud patch and panel, 8 days after being unwrapped and treated with 3 : 1 lanolin-beeswax mixture. Note that the bud patch is in good condition and that the surrounding cambium on the panel has survived. × about 1.

the American Tropics has shown that such losses may be as high as 75 percent.

As a preliminary step in determining the effect of lanolin on bud survival after unwrapping, several buds were top worked in large trees. On opening, half of the bud patches and the surrounding exposed cambium on the stocks were smeared with a mixture of 3 parts of lanolin and 1 part of beeswax, which was soft enough at air temperature for application with a finger. The other buds in the test were left untreated. During almost daily examinations that followed the unwrapping of the buds it was observed that when the buds were treated there was no discoloration of the cambial tissue surrounding the patch, which remained green, whereas the exposed cambium surrounding untreated patches began to darken within a few hours and became increasingly darker for several days. In some cases this tissue became spotted with mold. These conditions also prevailed in later buddings on *Hevea* seedlings (fig. 1). During this test it also was noted that the cambium around the treated buds grew so rapidly that it tended to grow over the edges of the patches 4 or 5 weeks after opening. Had the buds been allowed to remain dormant an equal number of months, no doubt some of them would have been completely overgrown.

USE OF LANOLIN MIXTURE ON BUDDINGS IN NURSERIES

The test of the lanolin-beeswax mixture on top-work buddings was not large enough to give conclusive proof, but it did show a definite advantage for its use on opened buds and led to the setting up of a more extensive experiment. One hundred seedlings were budded in the usual manner on May 22, 1945, and were unwrapped and examined 15 days later, on June 6, when it was found that all buddings had been successful and the bud patches were in excellent condition. As the buds were opened and examined, every other one was coated with a smear of 3 : 1 lanolin-beeswax mixture. Thus, there were 50 treated and 50 untreated buds. Examination of these buds on June 12 showed that 8 untreated buds were dead and all 50 of the treated ones were in excellent condition. On June 17 the treated buds were still in good condition, but 16 untreated ones were dead. Final examination was made on June 26, 20 days after opening, when 3 treated buds and a total of 18 untreated ones were dead. Thus, 94 percent of the treated buds remained alive in contrast to but 64 percent of those untreated.

Observations during this test suggested that some improvement might be obtained by applying the lanolin mixture at budding time in the fissure formed by the bulge of the flap covering the bud after its insertion. Wrapping of the bud with waxed tape was expected to force the lanolin under the flap and afford a protective covering to the exposed cambium of the stock surrounding the bud.

On June 12, 100 seedlings were budded; 50 of these were treated by the method just explained with the 3 : 1 lanolin-beeswax mixture and 50 were left untreated. When unwrapped 14 days later, all buds were in excellent condition. Of the 50 buds treated at budding, 25 were smeared again with the lanolin-beeswax mixture immediately on opening; the remaining 25 were not treated a second time. Of the 50

buds not treated at budding, 25 were smeared with the mixture on opening and the remaining 25 were left untreated as checks.

All treated buds, whether treated at budding, opening, or both, were in good condition when examined 8 days after unwrapping, whereas 6 of the untreated checks were dead. Final examination of these buds was made on July 31, 35 days after opening, when 5 of the buds treated at budding and again at opening and 5 of those treated only at budding were dead; 9 untreated check buds were lost, as compared with none for buds treated only at opening. When treated at budding apparently there was some forcing of the lanolin-beeswax mixture under the bud patch by the pressure exerted by the wrapping tape. From observations in subsequent tests, which gave the same indication, treatment at budding seldom equaled treatment at opening only as a means of reducing loss in *Hevea* buddings.

USE OF VARIOUS UNGUENTS ON BUDS

In order to observe the effects of applying unguents other than the lanolin-beeswax mixture to the bud-patch area, seedlings were treated at the time of budding as follows: 10 with a 3 : 1 vaseline-beeswax mixture, 10 with a 3 : 1 vaseline-paraffin mixture, 10 with ordinary automobile cup grease, 10 with a 3 : 1 lanolin-beeswax mixture, and 10 left untreated as checks. These buds were unwrapped after 14 days, when they were again treated with the same combinations. On opening, all buds with the exception of 1 that was treated with cup grease were found in good condition. Final examination 24 days after this opening showed that 7 check buds, 1 treated with the vaseline-beeswax mixture, 2 with the lanolin-beeswax mixture, 9 with cup grease, and 1 with the vaseline-paraffin mixture were dead. The more damaging effect of cup grease was attributed to its soft consistency and the greater ease with which it was forced under the bud patch when pressure was applied with the budding tape.

To determine the effect of these same materials when applied on buds at the time of opening and not at the time of budding, 85 additional seedlings were budded. When unwrapped in 14 days, all were found to be in good condition and all were immediately treated with the same materials as used in the preceding test; there were 17 buds in each of 5 treatments. Final examination of the buds, 25 days after opening, showed that 7 untreated checks and 1 treated with cup grease were dead. All buds treated with lanolin-beeswax, vaseline-beeswax, and vaseline-paraffin mixtures were in perfect condition. From this it appears that any one of many inexpensive nontoxic materials of the proper consistency possibly might be applied with equal success as a protective coating over the exposed cambial tissue upon unwrapping the bud, but with the limited stock material at the disposal of the writers more extended tests were impossible.

USE OF LANOLIN MIXTURE ON BUDS FROM STORED BUDWOOD

As the foregoing experiments were conducted with freshly cut budwood, it was considered advisable to test the treatments on buds taken from budwood that had been stored for various periods under conditions approaching those encountered during long-distance shipments.

A quantity of budwood was cut on June 27 and divided into 2 equal lots. Each lot was carefully packed in slightly damp sphagnum moss and wrapped in kraft paper. Both packages were stored indoors at air temperature. After 5 days 1 of the packages was opened and from the budwood 50 seedlings were budded. Half of these were treated with 3:1 lanolin-beeswax mixture at budding, and the other half were left untreated as checks. When the buds were opened 14 days later, all were found to be in excellent condition and the treated ones were given a second application of the mixture. Twenty-four days later it was found that only 3 of the treated buds had died in contrast to 8 of the check ones.

The second package of budwood was opened after a 10-day storage period and used in budding 50 seedlings which were treated in the same manner as those in the 5-day storage test. When opened after 14 days, 2 of the 25 check buds were dead but all the treated ones were in good condition. Twenty-four days after opening, a total of 11 of the original 25 check buds and 9 of the 25 treated with the lanolin-beeswax mixture at budding were dead.

At the time these two tests were begun the relation of treating at budding to treating only at opening had not been established, but it was apparent before their completion. Accordingly, another storage test was begun for the inclusion of both these types of treatment and the storage periods were increased to 10 and 20 days, respectively, as 5-day storage had appeared to have no definite aging effect on the budwood.

After the budwood had been stored 10 days, buds were inserted in 84 seedlings and every third bud was treated with the 3:1 lanolin-beeswax mixture. The remaining 56 buds were divided alternately into 2 groups of 28 buds each, 1 group being marked for treatment at opening and the other group for leaving untreated throughout the experiment as checks. Upon unwrapping, 14 days after budding, 1 check bud, 2 buds to be treated at opening, and 1 bud treated at budding were dead. Immediately after the unwrapping of these buds the 27 living originally treated were treated again. Through an error 25 instead of the 26 living buds to be treated at opening were smeared with the lanolin-beeswax mixture. This automatically added 1 bud to the untreated check group.

Of the 28 check buds alive at opening 17 (60.7 percent) were in good condition at the final examination, 24 days after unwrapping; of the 25 buds alive at opening and treated with lanolin at that time 21 (84.0 percent) were in good condition; of the 27 buds treated twice with lanolin and alive at opening 21 (77.8 percent) survived.

From budwood that had been stored 20 days 60 seedlings were budded. Through an oversight an unequal division was made of the seedlings between treatments, as 20 buds were treated with 3 : 1 lanolin-beeswax mixture at budding, 18 were held for treatment at opening, and 22 were maintained as untreated checks. When unwrapped 14 days later, 18 of the treated buds, 15 of those held for treatment at opening, and 19 of the check buds were alive. The 18 buds treated at budding were treated a second time at opening. When final examination of the buds alive at opening was made 24 days later, it was found that 13 (72.2 percent) of those treated at budding and again at opening were alive; 11 (73.3 percent) treated

only at opening were alive; and only 11 (57.9 percent) of the checks had survived.

EFFECT OF TIME OF TOPPING STOCKS ON BUDS TREATED WITH LANOLIN MIXTURE

As a final experiment in the use of lanolin as a means of reducing loss in *Hevea* buddings 100 additional seedlings were budded on August 2. These were unwrapped after 14 days, and all were found to be in good condition. At opening, 50 were smeared with the 3:1 lanolin-beeswax mixture and 50 were not treated. Stocks of half of each group of 50 were immediately cut off in the usual manner to force bud growth and the cut surface of the stock was painted with the 1:1 lanolin-beeswax wound mixture. Thus, 50 stocks into which 25 check buds and 25 treated buds had been inserted were cut back, but the remaining 50 were not cut. All uncut stock plants with living buds were cut back 12 days after opening to force the buds. By this time 11 of the previously uncut checks, 7 cut checks, and 1 cut lanolin-treated bud had died. Final examination was made 25 days after opening, when 11 of the checks not cut back at opening, 9 cut checks, 3 uncut lanolin-treated buds, and 1 cut lanolin-treated bud were dead. The results of this limited experiment indicate that seedlings containing living bud patches may, when unwrapped, be cut back immediately to force sprouting without fear of serious loss if the patches and surrounding cambium are treated with lanolin-beeswax mixture at the same time. In this and other experiments where a delay of 10 or more days occurred between the unwrapping of the bud and the cutting back of the stock, survival of bud patches treated with the lanolin-beeswax mixture at opening was greater than that of untreated buds.

EFFECT OF LANOLIN MIXTURE ON SPROUTING OF BUDS

An observation in connection with the immediately preceding experiment and one other involving the cutting back of stocks to force bud growth was the retarding effect the application of the 3:1 lanolin-beeswax mixture had on the growth of the buds.

In the experiment of May 22 in which 50 buds were treated with the 3:1 lanolin-beeswax mixture at opening and 50 were left untreated as checks, all living buds had the stocks above them cut back 11 days after opening to force the buds. Nineteen days after the cutting back of the stock 37 of the 47 surviving lanolin-treated buds were either swelling or sprouting, but only 1 had produced a shoot more than 1 mm. long; 26 of the 32 check buds were either swelling or sprouting, and 8 of these had shoots more than 1 mm. long. Final examination, 34 days after the cutting back of the stocks, showed all lanolin-treated and all check buds to be swelling or to have shoots. All but 1 of the check buds had shoots with an average length of 282.4 mm., whereas only 34 of the 47 lanolin-treated buds had produced shoots and these had an average length of only 91.4 mm.

The same retarding effect was observed in the experiment begun on August 2 in which 50 stocks were topped as soon as the buds were unwrapped and the remaining 50 were cut back 12 days after un-

wrapping. As reported for that experiment, half of the buds in each group had been treated with the 3:1 lanolin-beeswax mixture at opening whereas the others were left untreated as checks. In the seedlings cut back when the buds were opened, 4 of the 18 living untreated check buds were sprouting 12 days later when the other seedlings were topped, while only 3 of the 24 buds treated with lanolin mixture were sprouting.

At the end of this experiment, 25 days after the buds were unwrapped, 14 (87.5 percent) of the 16 living check buds in stocks cut back at opening were sprouting and 14 (58.3 percent) of the 24 living lanolin-treated buds in correspondingly cut-back stocks had sprouted. In the seedlings cut back 12 days after the buds were unwrapped 7 (50 percent) of the 14 living check buds had sprouted, whereas only 7 (31.8 percent) of the 22 living lanolin-treated ones had done so.

SUMMARY

Wounds arising from the pruning of *Hevea* rubbertrees kept in better condition and healed more rapidly when treated with 1:1 lanolin-beeswax mixture than when treated with any other wound dressing.

Buds from fresh budwood or from that stored for periods up to 20 days, when set in the tops of mature trees or in the customary location in seedlings, survived in much greater numbers when the bud patch and the surrounding area of cambium on the stock were treated with 3:1 lanolin-beeswax mixture at opening, or unwrapping, than did untreated buds.

Single treatment with the 3:1 lanolin-beeswax mixture at time of budding or treatment at time of budding and again at opening seldom was found to be as beneficial as treating only at the time of unwrapping the buds. Several other unguents that gave material benefit when applied at opening of the buds were used, but the softest of these caused increased mortality to buds when applied at time of budding.

Applications of lanolin-beeswax mixtures to bud patches and surrounding cambium and to the cut surface of the stock may allow cutting back of the stock at the time of unwrapping of the buds with negligible loss, thus eliminating the usual waiting period of 10 to 14 days between opening of the buds and cutting back of the stock during which under present practice the death or survival of the buds is determined.

Buds treated with the 3:1 lanolin-beeswax mixture were found to sprout more slowly after cutting back of the stocks than did untreated ones.

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